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AN APPROACH TO THE SYNTHESIS OF α-L-FUCOPYRANOSYL PHOSPHORIC MONO- AND DIESTERS VIA PHOSPHITE INTERMEDIATES

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Abstract: The reagent chloro-β-cyanoethyl-N,N-diisopropylamino-phosphoramidite reacts smoothly with the anomeric hydroxyl group of a properly protected (benzyl) α-L-fucopyranose to afford a relatively stable phosphite intermediate in high yield. The latter can easily be converted into valuable α-L-fucopyranosyl phosphoric mono- and diesters.

In naturally occurring phosphoric acid esters of oligosaccharides the ester linkages between the hydroxyl groups of the sugar moiety and phosphoric acid may either consist of one anomeric or solely non-anomeric hydroxyl functions. The formation of the latter type of phosphate esters can easily be accomplished by the well-established phosphotriester methodology developed for the preparation of nucleic acids. For example, the preparation of non-anomeric phosphodiester bonds present in teichoic acids or glycophospholipids has been published recently[1,2]. The synthesis, however, of glycosyl phosphates by a direct phosphorylation of an alcohol group at the anomeric centre is far less advanced[3].

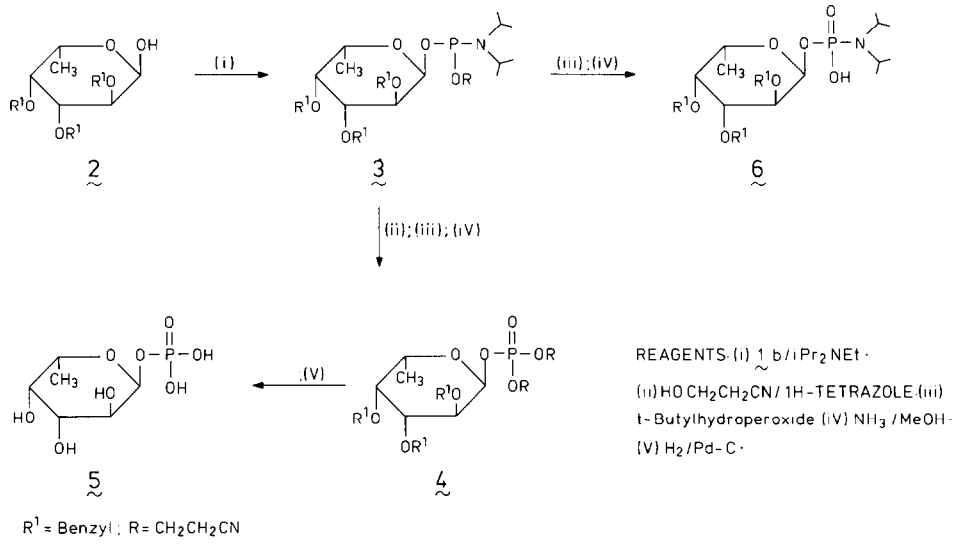
We now report that the phosphitilating reagent 1b, as developed by Sinha et al.[4] for the solid-support synthesis of DNA, can be used successfully for the preparation, *via* phosphite intermediates (e.g., 3), of anomeric phosphates of sugars.

Up to now, only one of the many phosphitilating reagents developed in nucleic acids chemistry has been adopted for the preparation of glycosyl phosphates. Thus, the synthesis of the latter compounds was accomplished, for the first time, by Ogawa et al.[5] using the bifunctional reagent 1a[6]. However, attempts[7] to phosphitilate the anomeric hydroxyl group of a Lipid A derivative by the same reagent failed[8].

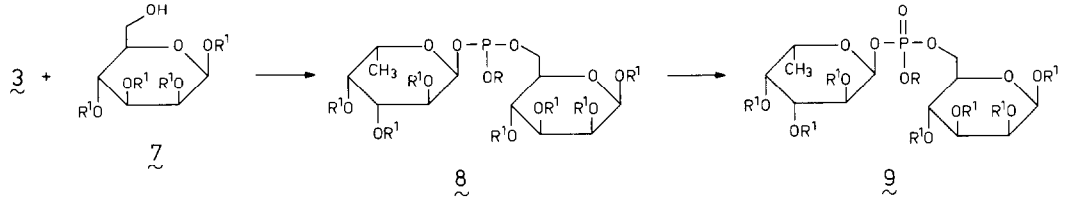
1 a: X = Cl; R = CH_2CCl_3.
b: X = $N(CHMe_2)_2$, R = CH_2CH_2CN.
c: X = $N(CH_2)_4O$; R = $CHMeCH_2CN$.
d: X = $N(CH_2)_4O$; R = $C(Me)_2CH_2CN$.
e: X = $N(CH_2)_4O$; R = $CH_2CH_2SO_2Me$.

The advent of a new type of reagents (i.e., 1b, 1c[9], 1d[9] and 1e[10]), of which the protective group at phosphorus (V) can be removed by mild base treatment, urged us to study the applicability of these reagents towards the preparation of glycosyl phosphates. Further, we selected 1b, on the ground of its easy accessibility, as the phosphitilating reagent.

The synthesis of the key intermediate 3 was performed as follows. To a stirred solution of 1b (1.3 mmol) and iPr_2NEt (1.95 mmol) in dry CH_2Cl_2 (5 ml), in a dry N_2-atmosphere, was added 2,3,4-tri-O-benzyl-α-L-fucopyranose[11] 2 (1 mmol). TLC-analysis of the reaction mixture, after 20 min at 20°C, revealed the absence of 2 and the formation of a product with a higher R_f-value. The solution was diluted with CH_2Cl_2 (5 ml) and washed with ice-cold aqueous solutions of $NaHCO_3$ (10%; 10 ml), satd. NaCl (3 x 20 ml) and water (20 ml). The organic layer was dried (Na_2SO_4) and the filtrate was concentrated to afford crude 3 as a colourless oil in

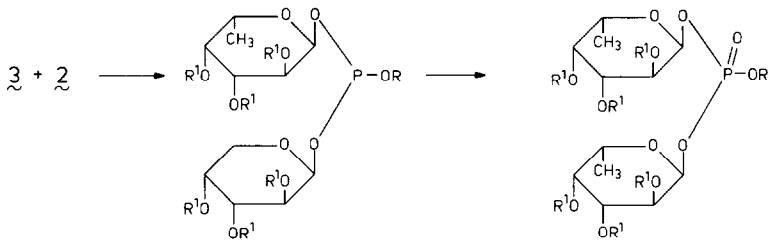


a yield of 97%. NMR-spectroscopy[12] of 3 thus obtained indicated that the product was contaminated with a small quantity of hydrolyzed 1b. Crude 3 was now converted in a one-pot procedure (steps ii, iii and iv, respectively) into the α-L-fucopyranosyl phosphate 4 ($R=Na^+$). To a stirred solution of crude 3 (0.5 mmol) in dry acetonitrile (2.5 ml) was added β-cyanoethanol (0.09 ml) and 1H-tetrazole (1.0 mmol) in CH_3CN (2 ml). ^{31}P-NMR spectroscopy of the reaction, after 20 min at 20°C, showed complete conversion of 3 [δp (CH_3CN) 150.9 and 150.1] into the expected di-β-cyanoethoxy phosphite [δp (CH_3CN) 140.4 (95%α) and 139.8 (5%β)]. The latter was oxidized with t-butylhydroperoxide[13] (0.17 ml) and left for 1 h at 20°C. ^{31}P-NMR showed the formation of 4 [δp (CH_3CN) -2.3] to be complete. The removal of the two β-cyanoethyl groups was effected by adding a cold and saturated solution of ammonia in methanol (4 ml) to the reaction mixture. The solution was stirred for 3 h at 0-5°C, and concentrated to a small volume. Crude 4 (R=H) was purified by Sephadex LH20 chromatography and converted (Dowex 50W Na^+-form) into the sodium salt, to afford homogeneous[12] 4 [$R=Na^+$; δp (MeOH) -1.43; yield 89% (based on 2)] as a solid. Hydrogenolysis of 4 ($R=Na^+$) over Pd/C gave, after purification, α-L-fucopyranosyl phosphate 5 the analytical data (^{31}P- and ^{1}H-NMR) of which were in accordance with those reported earlier[14]. Key intermediate 3 was now converted by oxidation (step iii), followed by ammonolysis (17 h at 20°C), to afford, after purification, homogeneous 6 [Na^+-salt; δp ($CHCl_3$) 5.98] in a high yield.



Further, coupling of 3 (0.5 mmol) with benzyl-2,3,4-tri-O- benzyl-β-D-mannopyranoside[15] 7 (0.6 mmol) in the presence of 1H-tetrazole (1.0 mmol) gave, as evidenced by ^{31}P-NMR, mainly 8 [δp (CH_3CN) 141.1 and 140.1]. The latter was, without further isolation, oxidized (step iii) to give fully-protected 9 [δp (CH_3CN) -1.72 and -2.15] which, after deblocking (step iv) and purification, afforded homogeneous[12] 9 [R=Na^+; δp ($CHCl_3$) -2.09] in a yield of 76% (based on 3).

At this stage of our study, we were interested to find out if the phosphitilation approach could also be applied towards the preparation of 11 (R=Na^+) in which two anomeric centres are part of the phosphodiester bond. To our knowledge, the occurrence in nature of this type of linkage has never been proved. Monitoring of the coupling of 3 with 2, which was realized under the same conditions as used for the preparation of 8, showed nearly complete formation of 10 [δp (CH_3CN) 139.89]. The oxidation of 10 to yield 11 [δp (CH_3CN) -3.66] proceeded also very efficiently. However, ammonolysis[16] of the β-cyanoethyl group from 11 was



10 11

accompanied by fission of the phosphate linkage. Nonetheless, pure[12] 11 [R=Na^+; δp ($CHCl_3$) -4.44] could be isolated, after purification, in a yield of 40% (based on 3).

The results presented in this paper indicate that the monofunctional phosphitilating reagent 1b promises to be a useful tool for the preparation of biologically important glycosyl phosphates. An additional advantage of the use of reagent 1b is the easy accessibility of valuable intermediates. For example, compound 6, or its N-morpholino analogue[17], may be further used for the preparation of glycosyl pyrophosphates. On the other hand, compound 4 (R=H) can be coupled, as recently reported by Srivastava et al.[18], with 7 to afford 9 (R=Na^+). Finally, it has to be mentioned that we did not observe any anomerization in the phosphorylation process (e.g., conversion of 2 into 4). The latter finding entails that the anomerically purity of the final glycosyl phosphates will be determined mainly by the starting product. At present, we are studying in detail the feasibility of preparing properly-protected and anomerically pure starting compounds.

REFERENCES AND NOTES

1. Teichoic acids: J.J. Oltvoort, C.A.A. van Boeckel, J.H. de Koning and J.H. van Boom, Recl. Trav. Chim. (Pays-Bas), 101, 87 (1982). C.A.A. van Boeckel, G.M. Visser, J.P.G. Hermans and J.H. van Boom, *ibid*, 102, 526 (1983). J.J. Oltvoort, H. Kloosterman, C.A.A. van Boeckel and J.H. van Boom, Carbohydr. Res., 130, 147 (1984).
2. Glycophospholipids: C.A.A. van Boeckel, J.J. Oltvoort and J.H. van Boom, Tetrahedron, 37, 3751 (1981). C.A.A. van Boeckel and J.H. van Boom, *ibid*, 41, 4545, 4557 and 4567 (1985).
3. To our knowledge, only one example, in which the phosphorylation of an anomeric hydroxyl was achieved in a one-step procedure, has been published (see Ref. 14).
In most cases, the hydroxyl group was prior to its phosphorylation converted by one of the

folowing procedures into a : halogenose [M.L.Wolfrom et al., J. Am. Chem. Soc.,63,1050 (1941)]; 1,2-ortho ester [M.A. Salam et al., Carbohydr. Res., 103, 139 (1982)]; 1,2-oxazoline [C. Augé et al., Carbohydr. Res., 82, 85 (1980) and references cited therein]; 1-O-thallium salt [A. Granata etal., Carbohydr. Res., 94, 165 (1981)]; 1-O- lithium salt [M. Inage et al., Chem. Lett., 1281 (1982)]; 1-O-trichloroacetimidate [R.R. Schmidt et al., Tetrahedron Lett., 23, 405 (1982)]. The above two-steps procedures ensure in most cases the anomerically purity of the final glycosyl phosphates.

4. N.D. Sinha, J. Biernat, J. McManus and H. Köster, Nucleic Acids Res., 12, 4539 (1984).
5. T. Ogawa and A. Seta, Carbohydr. Res., 110. C2, (1982).
6. R.L. Letsinger and W.B. Lunsford, J. Am. Chem. Soc., 98, 3655 (1976).
7. C.A.A. van Boeckel, J.P.G. Hermans, P. Westerduin, J.J. Oltvoort, G.A. van der Marel and J.H. van Boom, Recl. Trav. Chim. (Pays-Bas), 102, 438 (1983).
8. Phosphitilation of the same molecule with the ditriazolide of 1a afforded the α-hydrogenphosphonate (for more details, see Ref. 7).
9. J.E. Marugg, C.E. Dreef, G.A. van der Marel and J.H. van Boom, Recl. Trav. Chim. (Pays-Bas), 103, 97 (1984).
10. C. Claessen, G.I. Tesser, C.E. Dreef, G.A. van der Marel and J.H. van Boom, Tetrahedron Lett., 25, 1307 (1984).
11. M. Dejter-Juszinski and H.M. Flowers, Carbohydr. Res., 18, 219 (1971).
12. The values of δp are expressed in p.p.m. downward from the external standard 85% H3PO4. 1H-NMR data: Compound 3; 5.41-5.0 (H1, complex, 2 diastereoisomers): Compoun 4 (R=Na^+); 6.01 (dd, H1), JH1-H2 3.412 Hz, JH1-P 7.59 Hz, 4.244 (dt, H2), JH2-H3 10.24 Hz, JH1-H2 3.412 Hz, JH2-P 3.20 Hz: Compound 6; 5.864 (dd, H1), JH1-H2 2.93 Hz, JH1-P 6.96 Hz: Compound 9; 5.764 (dd, H1'), JH1'-H2' 3.30 Hz, JH1'-P 6.88 Hz, 4.478 (s, H1): Compound 11; 5.746 (dd, H1), JH1-H2 2.95 Hz, JH1-P 6.91 Hz, 4.034 (dt, H2), JH2-H3 10.19 Hz, JH2-H1 2.95 Hz, JH2-P 2.95 Hz.
13C-NMR data: Compound 3; 94.90 (d, C1), JC1-P 20.51 Hz, 93.92 (d, C1), JC1-P 21.98 Hz: Compound 4; 94.05 (d, C1), JC1-P 5.86 Hz, 76.14 (d, C2), JC2-P 7.32 Hz: Compound 6; 94.15 (d, C1), JC1-P 7.33 Hz, 77.16 (d, C2), JC2-P 7.33 Hz, 46.40 (d, Cα-diisopropylamine), JCα-P 5.86 Hz, 23.19 (d, Cβ-diisopropylamine) JCβ-P 17.59 Hz: Compound 9; 101.18 (C1), 95.02 (d, C1'), JC1'-P 5.87 Hz, 77.06 (d, C2'), JC2'-P 8.79 Hz, 76.23 (d, C5), JC5-P 7.33 Hz, 65.59 (d, C6), JC6-P 5.86 Hz: Compound 11; 93.95 (d, C1), JC1-P 4.40 Hz, 76.08 (d, C2), JC2-P 4.42 Hz.
13. J. Engels and A. Jäger, Angew. Chem. Suppl., 2010 (1982).
14. H.A. Nunez, J.V. O'Conner, P.P. Rosevear and R. Bakker, Can. J. Chem., 59, 2086 (1981).
15. The synthesis of this compound will be published elsewhere.
16. At the moment a study of establishing the optimal conditions for the removal of goup R from 11 as well as the stability of 11 (R=Na^+) towards base is under investigation.
17. This compound was prepared by phosphitilation of 2 with the N-morpholino analogue of 1b (see Ref. 4), and further processing of the intermediate thus obtained according to the procedures described for the preparation of 6.
18. O.P. Srivastava and O. Hindsgaul, Carbohydr. Res., 143, 77 (1985).

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